**Alleviation of Myocardial Stunning by Leukocyte and Platelet Depletion**

William Westlin and Kevin M. Mullane, PhD

Neutrophils accumulate in myocardium rendered ischemic and reperfused. Activated neutrophils release mediators such as metabolites of oxygen that can compromise myocellular integrity and provoke cardiac dysfunction. Although it is established that leukopenia reduces infarct size, the role of leukocytes and the source of free radicals in posts ischemic contractile dysfunction is unresolved. A carotid left anterior descending coronary-artery extracorporeal circuit without \((n=8)\) or with a Leukopak filter \((n=6)\) to deplete the leukocytes and platelets from blood entering the left anterior descending artery was established in the anesthetized, open-chest dog 30 minutes before ischemia. Subendocardial segmental function was monitored by sonomicrometry, and ischemia was produced by stopping flow for 15 minutes followed by 3 hours of reperfusion. Depleting leukocytes by \(90\pm3.2\%\) and platelets by \(100\%\) improved segmental function \((30.5\pm7\% \text{ to } 74.1\pm12.7\%\) for control versus leukocyte-depleted dogs, respectively\) at 15 minutes of reperfusion. In the leukopenic group, however, there was a progressive decline in contractility to \(32.5\pm13.8\%\) by 3 hours of reperfusion that was associated with a return of leukocytes and, to a lesser extent, a return of platelets in the extracorporeal blood to \(70.2\pm21.9\%\) and \(15.5\pm4.3\%\) of systemic values, respectively. Removal of leukocytes and platelets from blood perfusing the coronary vascular bed only at reperfusion improved contractile function to \(67.7\pm6.9\%\) at 15 minutes and \(54.7\pm12.1\%\) at 3 hours \((n=6)\). Scanning electron microscopy revealed adherent leukocytes in the epicardial coronary arteries of control animals after 3 hours of reperfusion. During the period of reflow, retrograde coronary artery pressure, measured when antegrade flow was stopped for 30 seconds, was inversely correlated with the arterial leukocyte count \((r=0.77, p<0.02)\), whereas this relation was absent in leukopenic dogs \((r=0.15, p=NS)\). Thus, leukocytes accumulating in the posts ischemic myocardium may compromise coronary perfusion and the recovery of contractility, whereas removal of leukocytes at reperfusion attenuates myocardial stunning. *Circulation* 1989;80:1828–1836

Reperfusion of myocardium made ischemic for 5–20 minutes is associated with a prolonged depression of contractile function, termed the “stunned myocardium,” that occurs in the absence of myocellular necrosis.\(^{1,2}\) This functional abnormality is attributed to the reintroduction of molecular oxygen to the ischemic tissue rather than the reestablishment of flow per se. Recent studies using electron paramagnetic spin resonance techniques and spin-trap agents have demonstrated the formation of reactive oxygen metabolites when the heart is reoxygenated.\(^{3-8}\) That these oxygen-derived free radicals contribute to the posts ischemic contractile dysfunction is suggested by various studies demonstrating improved recovery of the stunned myocardium of dogs treated with agents that scavenge free radicals, including superoxide dismutase and catalase,\(^9-11\) N-2-mercaptopropionylglycine,\(^12\) captopril,\(^13\) or dimethylthiourea,\(^14\) or agents that prevent their formation, such as allopurinol,\(^15\) oxypurinol,\(^16\) or deferoxamine.\(^17,18\)

There are many potential sources of oxygen-derived free radicals in the reperfused heart. Intracellular sources include the electron-transport chain in mitochondria, the enzyme xanthine oxidase present in the vascular endothelium, and the oxygenases involved in eicosanoid production.\(^19\) Alternatively, extracellular sources include the auto-oxidation of catecholamines released at reperfusion or the polymorphonuclear leukocytes that accumulate in the reperfused myocardium.\(^19\) Leukocytes are an attractive source because they have the capacity to produce copious amounts of reactive oxygen species, and they also

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release other mediators that can influence cardiac function.\(^{19}\) Moreover, Engler and Covell\(^{20}\) found that leukocyte depletion improved posts ischemic recovery of function. This study has been criticized, however, because functional recovery in the presence and absence of leukocytes was compared within the same animal and, unusually, posts ischemic contractile function returned completely within 1 hour.

The purpose of the present study was to examine the role of leukocytes in myocardial stunning by removing leukocytes from the blood entering only the coronary vascular bed by the use of Leukopak filters. Three groups of dogs were studied—a control group with an extracorporeal circuit but lacking a filter, a group in which the blood was depleted of leukocytes and platelets before the ischemic insult, and a group in which the blood was passed through the Leukopak filter only during reperfusion. In addition, studies were undertaken to determine the mechanism by which leukocytes can compromise the posts ischemic recovery of function.

Methods

Experimental procedures complied with the “Guiding Principles in the Use and Care of Animals” approved by the Council of the American Physiological Society, as well as with state and federal laws, and were reviewed by an internal animal care committee. CIBA-GEIGY is accredited by the American Association of Accreditation of Laboratory Animal Care.

Surgical Preparation

Male mongrel dogs (14–21 kg) were anesthetized with thiamylal sodium (15 mg/kg) followed by α-chloralose (80 mg/kg i.v.) supplemented as needed. A cuffed endotracheal tube was inserted, and the animal mechanically ventilated with room air. Cannulae were inserted into the left femoral artery and vein to measure aortic pressure and for the administration of anesthetic and other agents, respectively. A separate cannula was inserted into the left carotid artery for use in the extracorporeal circulation.

A left thoracotomy was performed, and the heart was exposed and suspended in a pericardial cradle. The left anterior descending (LAD) coronary artery was cleared, and ligatures were placed around the vessel for later cannulation. A micromanometer-tipped catheter (7F, Gaeltec) was inserted into the left ventricle at the apex to measure left ventricular pressure (LVP) and its first derivative (LV dP/dt). Regional myocardial segmental function was quantified with pairs of ultrasonic dimension crystals placed in the subendocardium in the zone of the myocardium to be rendered ischemic.

An extracorporeal circuit was established between the left carotid artery and the LAD coronary artery (Figure 1). Blood from the cannula leading from the carotid artery was pumped by a Watson-Marlow roller pump either through a Leukopak filter (Fenwal Laboratories, Travenol Labs, Deerfield, Illi-

\[ n = \text{subjects per group} \]

Figure 1. Schematic of surgical procedure used in which an extracorporeal circuit was established between left carotid artery and left anterior descending (LAD) coronary artery in anesthetized open-chest dogs. LV, left ventricle.

\[ n = \text{number of survivors} \]

nois) to deplete the blood of leukocytes or through a bypass circuit, thereby allowing whole blood to enter the coronary artery. A pulse dampener was inserted between the pump and the LAD cannula to suppress pressure oscillations from the roller pump. An injection port was inserted distal to the leukocyte filter to withdraw blood samples for the determination of leukocyte and platelet counts and hematocrit (Cell-Dyn 900, Sequoia-Turner, Mountain View, California). Systemic blood samples were also obtained for evaluation of the effects of the Leukopak. Although granulocytes were not measured in the present study, Engler and associates\(^{20,21}\) have previously demonstrated that these Leukopak filters provide a greater loss of granulocytes than other white cell populations. The blood was then pumped through a cannula inserted into the LAD just distal to the first major diagonal branch. Heparin (500 units/kg) was administered immediately before initiating the extracorporeal circulation and supplemented (250 units/kg) every 1.5 hours.

At the conclusion of the experiment, the risk area was measured as a percentage of the total left ventricular after demarcation by the systemic administration of Evans’ blue dye while the LAD coronary circulation was perfused with normal saline.\(^{13}\) Ventricular fibrillation was induced by the administration of saturated potassium chloride, and the heart was immediately excised. The left ventricle was cut from apex to base into 0.7-cm-thick transverse sections, and the area at risk was traced onto clear acetate sheets. The sizes of the risk area and total left ventricle were determined by planimetry.\(^{13}\)

Protocol

When cannulating the LAD coronary artery, blood was pumped at a constant flow either through the bypass circuit (\(n = 11\)) or the Leukopak filter (\(n = 6\)) to allow whole blood or leukocyte-depleted blood to pass into the LAD arterial bed, respec-
Retrograde Pressure Measurements

In a subset of the control group (n=5) and groups with Leukopak (n=7), retrograde pressure distal to the site of coronary artery occlusion was determined. A sidestream pressure transducer was included in the extracorporeal circuit just proximal to the LAD cannula. The perfusion pump was stopped for a period of 30 seconds at 60, 120, and 180 minutes of reperfusion to determine retrograde pressure in the coronary artery.

Scanning Electron Microscopy

Sections of epicardial coronary arteries were perfusion-fixed for histologic analysis with Carson’s buffered formalin (62.0 mM NaH₂PO₄, 42.0 mM NaOH, 4% formaldehyde, pH 7.2) at 180 minutes of reperfusion. Vessels were stored at 5°C in fixative until prepared for histology. The tissue was cleared of any loose connective tissue, cut open, and pinned flat on Teflon plates. Tissues were placed in 1% osmium tetroxide for 2.5 hours, rinsed, and then placed in 1% aqueous thiocarbohydrazide for 1 hour. Samples were then rinsed, placed in 1% aqueous osmium tetroxide for an additional 2 hours, rinsed, and dehydrated in an ethanol series for 20 minutes each (35%, 50%, 70%, 80%, 95%, and 100%) followed by acetone for 20 minutes, and then dehydrated in a critical-point dryer. After the dehydration procedure, samples were fixed on stubs and sputter-coated with gold. Individual vessels were examined in a blinded manner by an investigator using a Philips SEM 515 scanning electron microscope. To determine the number of neutrophils present and the state of activation of these cells, samples were rated semiquantitatively as follows: 0, cells sparse or absent; 1, few cells present; 2, many cells present; and the incidence of cells with extruded pseudopods: 0, none; 1, few; 2, many or all. The presence or absence of neutrophil aggregates was also determined.

Analysis and Statistics

Segment shortening (SS), mean aortic blood pressure, LVP, LV dP/dt, and an electrocardiogram (limb lead II) were monitored continuously throughout the experiment on a Gould multichannel recorder. Hemodynamic measurements were determined from a mean of five continuous cardiac cycles. SS measurements were timed using LVP and LV dP/dt. End-diastolic length (EDL) and end-systolic length (ESL) were defined as the onset of the rapid rise in LVP and the peak negative dP/dt, respectively. EDL and ESL were measured from 10–12 continuous cardiac cycles for each sample period and averaged. To correct for variation in initial separation of crystal pairs, segment length measurements are expressed as a percentage of preocclusion (control) values. Percent SS was then calculated from the formula:

\[ \%SS = \frac{(EDL - ESL)}{EDL} \times 100 \]

The significance of differences through time or between groups for hemodynamic data and of differences in percent SS were calculated by repeated measures analysis of variance. The Dunnett’s test was used to compare control versus leukopenic groups. The histologic sections were evaluated semiquantitatively and analyzed by a Kruskal-Wallis test for nonparametric data. All values are given as mean±SEM.

Results

Exclusion of Dogs

Of the 24 dogs used in the present study, four dogs were excluded from the final analysis. In the control groups, two dogs were excluded due to a lack of passive bulging during ischemia, and one died from ventricular fibrillation at 1 minute of reperfusion. In addition, one dog was excluded from the group in which the Leukopak filter was instigated at reperfusion because of ventricular fibrillation at 1 minute of reperfusion. Thus, results are presented for eight control dogs, six depleted of leukocytes before ischemia, and six rendered leukopenic at reperfusion.

Extracorporeal Circulation

The time to establish the extracorporeal circulation averaged 2.5±0.2 minutes. SS was not different before or after establishing the circuit (100% versus 100.7±7.9% and 96.7±4.3% for control group and groups with Leukopak, respectively) (Table 1); neither were any other measured hemodynamic variables. In addition, in animals depleted of leukocytes, segmental function and other hemodynamic parameters were not altered before occlusion after depleting 90±3.2% of the leukocytes (100% versus 105.0±2.6%) (Table 1). The Leukopak filters also depleted the blood of platelets but did not change the hematocrit (Figure 2).

Segmental Function

The segmental-contractile function for control and leukopenic animals is depicted in Figure 3. In
control animals, there was a transition from active SS to a passive systolic lengthening of the myocardium during coronary artery occlusion ($-30.7 \pm 5.2\%$). At reperfusion there was a transient, partial recovery of contractile function that was followed by a prolonged depression of segmental function throughout the remainder of the experiment. In the leukocyte-depleted group there was a degree of passive bulging of the myocardium during ischemia similar to that seen in controls ($-44.7 \pm 10.7\%$). In contrast, at reperfusion there was a dramatic recovery of segmental function (to $74.1 \pm 12.7\%$ at 15 minutes of reperfusion). This functional improvement subsided, however, as the duration of reperfusion was prolonged, and by 150 and 180 minutes there were no significant differences between these two groups ($7.1 \pm 12.7\%$ versus $30.7 \pm 10.1\%$ at 150 minutes of reperfusion; $6.0 \pm 12.0\%$ versus $32.5 \pm 13.8\%$ at 180 minutes of reperfusion, control versus leukopenic animals). This progressive decline in postischemic contractile function over 3 hours of reperfusion in the leukocyte-depleted dogs was associated with a steady rise in the leukocyte count entering the LAD coronary arterial bed from the extracorporeal circuit (Figure 2). The arterial leukocyte count rose steadily during the reperfusion period, returning to approximately 70% of preischemic control levels by 3 hours of reperfusion.

Myocardial stunning in dogs depleted of leukocytes during only the reperfusion period was then determined. The results on myocardial contractility are depicted in Figure 4. By reperfusing the ischemic region with blood depleted of leukocytes, there was a dramatic recovery of contractile function ($67.7 \pm 6.9\%$ at 15 minutes of reperfusion) similar to that seen in the group rendered leukopenic 30 minutes before ischemia. Moreover, because the leukocyte filter was used 45 minutes later in this protocol, the improved contractility persisted for the duration of the reperfusion period ($7.1 \pm 12.7\%$ versus $50.9 \pm 9.3\%$ at 150 minutes and $6.0 \pm 12.0\%$ versus $54.7 \pm 12.1\%$ at 180 minutes, control versus leukopenic animals, $p<0.05$).

<table>
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<th>TABLE 1. Baseline Hemodynamics for Three Experimental Groups*</th>
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<td>Segment shortening normalized (%)</td>
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*Before and after establishing extracorporeal circuit, with or without Leukopak filter. There were no significant differences between groups (ANOVA). LVSP, left ventricular systolic pressure.

**Figure 2.** Plots of leukocyte and platelet counts and hematocrit of extracorporeal blood entering left anterior descending coronary artery in presence (○) or absence (●) of a Leukopak filter. C, Control (systemic values); Pex, 30 minutes postestablishment of extracorporeal circuit just before ischemic period.

**Figure 3.** Plot of segment shortening expressed as percentage of preocclusion values in dogs subjected to 15 minutes of ischemia and 3 hours of reperfusion. ■, Perfusion with whole blood (n=8); ○, Perfusion with leukocyte-depleted blood, beginning before ischemia (n=6).
FIGURE 4. Plot of segment shortening expressed as percentage of preocclusion values in dogs subjected to 15 minutes of ischemia and 3 hours of reperfusion. ■, Perfusion with whole blood (n=8); □, Reperfusion with blood depleted of leukocytes only during reperfusion (n=6).

The number of leukocytes in the blood distal to the leukocyte filter also increased toward the end of the reperfusion period, but the time at which the leukocyte number was restored to 35% of peripheral blood values was 56±9 minutes into reperfusion in the experiments in which the filter was used before the ischemic event, compared with 130±23 minutes when leukopenia was induced at reperfusion (p<0.05, Student’s t test). Pooling the results obtained from control and leukopenic dogs to examine the association between the arterial white cell count and postischemic SS reveals a significant relation between the two parameters (Figure 5).

Coronary Retrograde Pressure

Retrograde pressure was used as an index of collateral perfusion.22 Retrograde pressures were measured before occlusion and at 60, 120, and 180 minutes of reperfusion by stopping antegrade flow for 30 seconds. This brief period of ischemia did not alter contractile function at any time point in any animal. Preischemic retrograde pressure was 24.9±1.5 mm Hg (n=12) that, when normalized to arterial pressure, averaged 0.214±0.018 units. This pressure did not differ between control or leukopenic animals (25.2±2.2 versus 24.8±2.1 mm Hg, respectively). During reperfusion, retrograde pressures did not change significantly in control animals (27.6±4.1, 24.8±2.8, and 21.6±3.9 mm Hg at 60, 120, and 180 minutes, respectively) but declined in leukopenic dogs (13.1±2.6, 14.2±2.1, and 19.1±1.6 mm Hg at the respective time points, p<0.05, ANOVA). Distal coronary pressure in control animals is inversely related to the arterial leukocyte count (Figure 6). When leukocytes are removed, however, this relation no longer exists.

Histological Assessments

Sections of epicardial coronary arteries within the previously ischemic region of the heart were analyzed semiquantitatively for the presence of neutrophils and the incidence of a “ruffled” appearance to the leukocytes (pseudopods). The presence or absence of neutrophil aggregates was also determined. The results are depicted in Figure 7. Vascular segments of control dogs subjected to 15 minutes of ischemia and 3 hours of reflow contained many adherent cells, some of which showed ruffling and pseudopod formation. Dogs rendered leukopenic before ischemia had less adherent cells within the coronary arteries than in whole blood–perfused experiments, however, the state of activation of these adherent cells was not different, as indicated by the incidence of pseudopod formation. In contrast, histologic sections of coronary arteries from dogs made leukopenic at reperfusion showed both a reduced number of cells and fewer pseudopods (both, p<0.05, nonparametric analysis). Leukocyte aggregates were evident in vascular segments of all untreated (control) dogs but were observed in arteries of only one of the leukopenic animals in which leukopenia was induced before coronary artery occlusion. No aggregates were observed in the group with leukopenia at reperfusion.

FIGURE 5. Plots of relation between arterial leukocyte count and postischemic contractile function. Number of leukocytes was determined in systemic arterial blood in control dogs (●) and from filtered blood entering the coronary cannula in dogs with Leukopak (○). Panel A depicts relation after 60 minutes of reperfusion and Panel B depicts relation after 120 minutes in 5 control and 11 leukopenic animals.

FIGURE 6. Plot of relation between retrograde coronary pressure and coronary arterial leukocyte count during reperfusion in control and leukopenic dogs. Values were obtained after 60 (circles) and 120 (squares) minutes of reperfusion. Data from control animals (n=5) are shown by solid symbols (●, ■) and those subjected to the Leukopak filter (n=7), by open symbols (○, □).
Hemodynamic Variables

Coronary artery occlusion produced a small, non-significant decrease in LVP and peak negative dP/dt in control dogs, with no change in mean systemic arterial pressure or heart rate (Figure 8). Leukocyte depletion before occlusion was associated with a greater reduction of LVP and peak negative dP/dt during 15 minutes of ischemia (both, \( p<0.05 \) versus control, ANOVA) and a fall in mean arterial pressure and heart rate. Consequently, the rate-pressure product, an index of cardiac work, was lower in the leukopenic group during the ischemic period (\( p<0.05 \) versus control, ANOVA). These hemodynamic changes reversed rapidly at reperfusion (Figure 8). In contrast, dogs in which leukocytes were depleted at reperfusion did not show these ischemia-induced changes in cardiac function, whereas a drop in peak negative dP/dt at 15 minutes of reperfusion was the only difference to control animals observed during reperfusion (Figure 8).

Discussion

The central observation of this study is that using Leukopak filters to deplete leukocytes and platelets from the blood entering the coronary vascular bed abrogates myocardial stunning even when the depletion is effected solely at reperfusion. These results support and extend those of Engler and Covell,\(^20\) who also showed that depleting the blood of leukocytes before a period of ischemia alleviated the posts ischemic contractile derangements usually observed. Moreover, the subsequent return of leukocytes was accompanied by a decline in segmental shortening that was not observed when leukocytes were removed only during reperfusion, suggesting that myocardial stunning can be delayed and is not solely dependent on events occurring within the first few minutes of reperfusion. There are a number of potential mechanisms whereby removal of leukocytes could improve segment function during reperfusion.

The use of electron paramagnetic spin resonance (EPR) spectroscopy, either alone or in conjunction with spin-traps such as \( \alpha \)-phenyl N-tert-butylnitrone or 5,5-dimethyl-1-pyrroline \( N \)-oxide, has revealed that reperfusion of an ischemic or hypoxic heart results in the transient generation of oxygen-derived free radicals that peak 0.5–10 minutes after the readmission of oxygen.\(^3\) These free radicals have been implicated in the posts ischemic derangements in contractility because a variety of free radical scavengers including superoxide dismutase and catalase,\(^9\) N-2-mercapto-1-propionylglycine,\(^12\) captopril,\(^13\) or dimethylthiourea\(^14\) alleviate the stunned myocardium. Various sources of free radicals have been implicated in reperfusion injury including the enzyme xanthine oxidase,\(^15\) the electron-transport chain in mitochondria or polymorphonuclear leukocytes\(^20\) that accumulates in the ischemic region.\(^23\) EPR studies indicate free radical formation in isolated hearts perfused in the absence of leukocytes, which leads to the suggestion that the important source of these mediators is tissue derived or intracellular. There are various intracellular free radical scavengers including superoxide dismutase, glutathione peroxidase, and catalase, however, that can protect against the deleterious consequences of intracellular free radical formation.\(^19\) Polymorphonuclear leukocytes are powerhouses of free radical production and can overwhelm the limited extracellular protective mechanisms.\(^24\) Moreover, the addition of leukocytes to buffer-perfusing isolated hearts subjected to hypoxia and reoxygenation aggravates the loss of contractility during reoxygenation,\(^25\) an effect reversed by SOD.\(^26\) Thus, in the present

FIGURE 7. Bar graphs of leukocyte accumulation and activation (as evidenced by “ruffling” or pseudopod formation) in epicardial coronary arteries of control (c), leukopenic before occlusion (■), and leukopenic at reperfusion (□) groups (\( n=3-4 \) in each group) after 15 minutes of ischemia and 3 hours of reperfusion. Three to four sections were analyzed from each animal to provide a score for that animal (see “Methods” for score system). *\( p<0.05 \) versus control, Kruskal-Wallis nonparametric test.

FIGURE 8. Plots of changes in heart rate (HR), left ventricular pressure (LVP), and peak negative LV dP/dt during ischemia and reperfusion in control dogs (■) and those made leukopenic either before (■) or after (□) ischemic event. Mean values are depicted with SEMs omitted for clarity. C, Control values before establishment of extracorporeal circuit; Pex, 30 minutes postextracorporeal circuit incorporation; 15' occl, 15 minutes of occlusion. *\( p<0.05 \) versus control values, repeated means ANOVA and Dunn’s test.
Activated granulocytes can also release other mediators capable of influencing myocardial function, integrity, or both including platelet-activating factor (PAF), metabolites of arachidonic acid (AA), and proteolytic enzymes. PAF and leukotriene (LT) C₄ reduce contractility of isolated papillary muscles, whereas PAF also exacerbates myocardial damage induced by coronary artery occlusion in vivo. Moreover, interactions between different leukocyte-derived mediators may promote or exacerbate myocardial injury. PAF, AA, and LT₄, for example, enhance superoxide anion formation by neutrophils, whereas myeloperoxidase released from granules interacts with hydrogen peroxide and chloride ions to form the toxic hypochlurous acid (see reference 28 for review).

An alternative mechanism by which leukocytes may compromise postischemic contractility is by the plugging of small coronary arterioles and capillaries resulting in underperfusion (continued ischemia) of discrete regions of the myocardium. Engler and colleagues proposed that capillary plugging by neutrophils contributes to the no-reflow phenomenon. In the present study, neutrophils were observed within the large epicardial coronary vessel, adhering to the endothelium and migrating beneath the endothelial layer, whereas O'Neill and coworkers also observed neutrophil accumulation after myocardial stunning. Measurements of retrograde coronary pressure may support the idea that these activated neutrophils impair reflow. There is a linear relation between coronary pressure and collateral blood flow in the pressure range of 10–75 mm Hg. Moreover, we observed an inverse relation between arterial leukocyte counts and retrograde coronary pressure, that is, collateral flow is diminished when leukocyte numbers are elevated. Because coronary vasodilators also alleviate the stunned myocardium, it is plausible that compromising reflow by vascular plugging or lumen narrowing could contribute to the contractile derangements. The lower retrograde pressures in leukopenic dogs probably reflects a decrease in vascular resistance in the coronary vascular bed.

An additional means by which the Leukopak filters could improve postischemic contractility is by a reduction in blood viscosity secondary to leukocyte depletion, resulting in enhanced tissue perfusion. Neutrophils are large cells that must deform to pass through the microcirculation. The resistance imposed by one neutrophil is approximately 2,000-fold that of a red cell. Consequently, neutrophils can have a large impact on vascular resistance and organ perfusion.

Removal of platelets by the filters could potentially underlay a beneficial effect by an unknown mechanism. By contrast with ischemia-reperfusion-induced myocardial necrosis in which selective neutropenia reduces infarct size and thrombocytopenia does not, the role of platelets or platelet-derived mediators is not readily apparent. Moreover, platelet accumulation in the reperfused heart is considered secondary to a neutrophil-mediated event rather than representing a primary response. In the present study, the loss of platelets and their subsequent return did not correlate with changes in contractility (compare Figures 2 and 3). Whether platelet depletion can augment postischemic contractility, however, requires direct examination.

Finally, cardiac work during ischemia was significantly reduced in leukopenic dogs as denoted by decreases in LVP, peak negative dP/dt max, and the rate-pressure product, which was not observed in control animals. Decreasing the work of the heart could diminish the severity of the ischemic insult and result in an increasing recovery during reperfusion, independent of an effect on leukocytes. This mechanism, however, would not account for the improvements in postischemic contractility observed in dogs that were rendered leukopenic at reperfusion and showed no significant hemodynamic changes during ischemia. Rather, these results imply that events related to the presence of leukocytes at reperfusion are critical in determining the extent of the contractile derangements.

Although these present observations are in accord with the results of Engler and Covell, O'Neill and colleagues reported no functional improvement in dogs rendered neutropenic with a specific antiserum. The degree of leukocyte depletion in this latter study and the present one were similar at about 90%. It can be argued that passing blood through an extracorporeal circuit activates the leukocytes and platelets, thereby inappropriately enhancing their contribution to the stunned myocardium. Postischemic SS, however, was not significantly different between control animals in the present study and those in an earlier study without an extracorporeal circuit. The trapping of leukocytes by the Leukopak filters may lead to cell activation with the release of leukocyte-derived agents that improve contractility, but the Leukopak filters did not influence contractility of the preischemic heart. Moreover, a 20-second delay circuit was interposed between the filter and the coronary circulation to permit inactivation of any labile substances released. Heparin used to anticoagulate the blood increases extracellular superoxide dismutase concentrations and could improve postischemic function, although control animals were also heparinized and no significant return of function was observed. Using an antiserum to deplete leukocytes must be done cautiously because the injection of foreign serum or
blood produces a “shock-like” reaction typified by systemic hypotension, an elevation in central venous and pulmonary arterial pressures, and activation of white blood cells and platelets. Changes in endothelial cell function, including adhesion and infiltration by monocytes, have also been reported. Thus, the injection of heterologous antiserum to induce neutropenia may exert other, potentially deleterious effects. The full consequences of either procedure (antiserum or filters) remain unknown and may account for some of the differences in functional recovery. While this manuscript was under review, however, Jeremy and Becker published a similar study using an extracorporeal circuit in which leukopenia and thrombocytopenia was induced by filtering the blood but which did not result in amelioration of posts ischemic dysfunction. Although there are a number of technical differences between this study and our own, for example, choice of anesthesia, constant pressure versus constant flow perfusion systems, Sepacell versus Leukopak filters, and with or without volume expansion with 10% dextran, there are two major differences that could account for the discrepancy. First, Jeremy and Becker used a 10–minute period of ischemia in contrast to our 15-minute period of ischemia. The shorter ischemic period resulted in a greater-than-50% return of function by 20 minutes of reperfusion in six of seven control animals compared with approximately 20–30% in the present study. Thus, less severe levels of posts ischemic injury may not have been sufficient to activate neutrophils. Moreover, a significant improvement in function may be harder to discern using any therapeutic intervention in this setting in which contractility is restored to more than 50% in controls. Second, the dogs used in the protocol of Jeremy and Becker had control leukocyte and platelet counts equal to almost one third of the values observed in the animals used in this study. This partial leukopenia-thrombocytopenia could obscure any beneficial effects of the filter. Extrapolating functional recovery versus leukocyte counts (Figure 5) indicates that the number of white blood cells in the dogs of Jeremy and Becker (approximately 3,500/µl) would give an approximate 53% return of contractile function, which compares very favorably with the approximate 55% return observed in six of seven dogs. Thus, the variation may be attributable to different baseline values for the blood cells and actually not represent opposing results.

The present study indicates that filtration of the coronary arterial inflow blood by Leukopak filters enhances the recovery of posts ischemic contractility. This functional improvement appears to correlate with removal of leukocytes but may also reflect additional effects of the filters.

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